Targeting toll-like receptor 7/8 improves host anti-infective response in alcoholic cirrhosis

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COMMENTARY

TARGETING TOLL-LIKE RECEPTOR 7/8 IMPROVES HOST ANTI-INFECTIVE RESPONSE IN ALCOHOLIC CIRRHOSIS

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The production of reactive oxygen species (ROS) by activated macrophages and neutrophil granulocytes represents an important cause of alcohol-induced oxidative stress and significantly contributes to the pathogenesis of alcoholic liver disease (ALD) [1]. Furthermore, diffuse neutrophil infiltration of the liver is a key feature of acute alcoholic hepatitis actively participating to parenchymal injury [2]. However, during ALD progression to cirrhosis, neutrophil functions such as ROS production, bacterial phagocytosis and granule exocytosis are impaired leading to an increased susceptibility to bacterial infections of cirrhotic patients [3]. Indeed, the development of bacterial peritonitis is a common complication of cirrhosis, while sepsis is a major cause of mortality of patients with decompensated alcoholic cirrhosis being also associated with multi-organ failure and immune deficiencies [4].

The key role of neutrophils in anti-bacterial defences mainly relies on the capacity of the enzyme NADPH oxidase 2 (NOX2) to generate superoxide anion (O_2^-) during a process known as oxidative burst. Superoxide anion, in fact, by conversion to hydrogen peroxide fuels myeloperoxidase-mediated production of the powerful bactericidal agents, hypochlorite and chloramine [5]. NOX2 is a multi-protein enzymatic system consisting of the transmembrane proteins gp91^{phox} and p22^{phox} forming the catalytic unit flavocytochrome b558 and of several cytosolic components, including the p67^{phox}, p47^{phox}, p40^{phox} and Rac1/2 sub-units [5]. Upon phosphorylation of p47^{phox}, the different sub-units assemble to the plasma membrane forming the active enzyme. Thus, an efficient ROS production requires an optimal expression of all the NOX2 components and coordinated signals driving the enzyme assembly [5].

In this issue, Rolas and co-workers [6] add further insights into the mechanisms responsible for the deficit in neutrophils' bactericidal activity that characterizes alcoholic cirrhosis by investigating some of the mechanisms responsible for the impairment of ROS production by NOX2. They report that the deficiency in oxidative burst that characterizes the response to formylated peptides (f-MLP) of neutrophils from decompensated cirrhotic patients was associated with a severe reduction in the intracellular content of gp91^{phox}, p22^{phox} and p47^{phox}, despite their unchanged mRNA levels. By investigating the mechanisms involved they observed that the decrease of gp91^{phox} was partially due to an enhanced protein degradation due to elastase released by neutrophils themselves and more abundant in the plasma of cirrhotic patients. In addition, the lowering of NOX2 components also involved an impairment of protein synthesis consequent to the reduction of the kinase mammalian target of rapamycin (mTOR), a key regulator of cellular anabolic processes. Interestingly, treating the neutrophils from cirrhotic patients with the Toll-like receptor-7/8 (TLR-7/8) agonists CL097 and R848 restored ROS production by stimulating the synthesis of NOX2 components through a process involving mTOR activation. TLR-7/8 agonists were also active when added to whole blood, while the use of the elastase inhibitor N-(Methoxysuccinyl)-Ala-Ala-Pro-Val-chloromethylketone (NEI) had a minimal impact on NOX2 recovery. The effect of TLR agonists was rather specific, as the TLR-4 stimulation by lipopolysaccharide enhanced gp91^{phox} expression, but modestly improved ROS production. Such a specificity might likely relate to the capacity of TLR-7/8-mediated signals to potentiate NOX2 activity through the phosphorylation of p47^{phox} by protein kinase B (PKB; AKT), mTOR and p38 mitogen activated kinase (p38MAPK) [7] (Fig. 1). From these data, the authors conclude that elastase-dependent degradation and mTOR impairment contributes to the decrease gp91^{phox} and ROS production observed in patients with alcoholic cirrhosis. However, the latter process appears be more relevant to explain the defect in ROS production, as protein synthesis can compensate for the loss of gp91^{phox} due to elastase-mediated degradation. A deficit of gp91^{phox} also accounts for the lowering in ROS-mediated bacterial killing by monocytes of patients with alcoholic hepatitis [8], indicating that in alcohol abusers similar mechanisms can be responsible for affecting the functions of different phagocytic cells. Interestingly, TLR-7/8 stimulation of AKT/mTOR also contributes to restore neutrophil's bactericidal activity by promoting myeloperoxidase release [9] (Fig. 1). Nonetheless, several issues remain still open. For instance, little is known about the mechanisms that lead to the lowering of neutrophil mTOR in advanced ALD and it is still unclear how TLR-7/8 stimulation acts in reverting mTOR activity. On the other hand, the

presented data add to the consensus that mTOR inhibitors are most likely ill-advised as anti-fibrotic treatment in advanced liver fibrosis, as they will inevitably worsen the defective oxidative burst capacity of neutrophils and thus increase the risk of infections.

In conclusion, this work nicely supports previous data from the same group pointing to a key role of AKT/mTOR in the mechanisms affecting the bactericidal activity of neutrophils in alcoholic cirrhosis [7,9]. The significance of the presented data mainly derives from its possible clinical translation into using TLR-7/8 agonists to reverse alcohol-mediated impairment of neutrophil ROS production, and thus improve the host's antibacterial defence of patients with alcoholic (and non-alcoholic?) cirrhosis. Whether this could be implemented in a treatment of established infections or in their prevention remains to be determined. However, as a novel therapeutic target, TLRs clearly deserve further investigation in humans, particularly, since prophylactic antibiotic treatment in patients with cirrhosis achieves only a moderate benefit [10]. In this regard, it seems important to emphasize that TLR agonists have been shown to be effective in promoting both innate and adaptive immunity [11] and their use might exert a synergistic therapeutic effect with that of granulocyte colony-stimulating factor [12] in controlling infective complications of advanced ALD.

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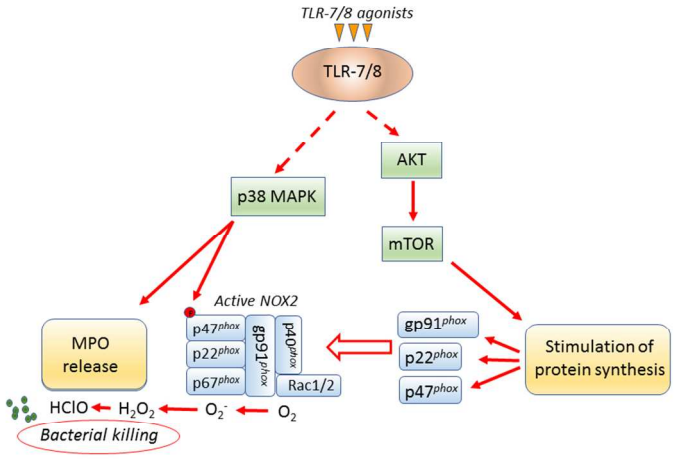
Figure caption

Figure 1: Proposed mechanisms by which TLR-7/8 stimulation improves the antibacterial activity of neutrophils from patients with alcoholic cirrhosis. Abbreviations: AKT protein kinase B; NOX2 NADPH oxidase 2; MPO myeloperoxidase; mTOR mammalian target of rapamycin; TLR-7/8 Toll-like receptor-7/8; O_2^- superoxide anion; H_2O_2 hydrogen peroxide; HClO hypochlorous acid; p38MAPK p38 mitogen activated kinase; NOX2 NADPH oxidase 2; $gp91^{phox}$, $p22^{phox}$ $p67^{phox}$, $p47^{phox}$, $p40^{phox}$, Rac1/2 NOX2 sub-units.



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